ENGINEERING MONOCLONAL ANTIBODIES TO IMPROVE STABILITY AND PRODUCTION TITER

RELATED APPLICATIONS

[0001] The present application claims the benefit of U.S. Provisional App. No. 62/787,867 filed Jan. 3, 2019, which is incorporated by reference in its entirety herein.

FIELD

[0002] The presented subject matter relaters to the field of protein engineering. Specifically, the presented subject matter relates to engineering antibodies, especially monoclonal antibodies, and variants thereof, to improve their stability and production.

BACKGROUND

[0003] Monoclonal antibodies (mAbs) that are recombinantly produced (and active fragments thereof) are important therapeutic tools. However, as these molecules are complex, many challenges need to be met to facilitate production, storage, and therapeutic administration of these molecules.

[0004] Two challenges concern production and stability. mAbs are produced in bioreactors from engineered cells, such as Chinese Hamster Ovary (CHO) cells. However, production levels can be low and can vary between mAbs. Low production levels increase production costs, including production time, labor, and consumed resources, such as the necessary components for operating the bioreactor. Furthermore, a lack of stability impacts the "shelf-life" of mAbs. Degraded mAbs can be less potent, and fragmented mAbs can present an immunologic risk.

[0005] Therefore there is a need to improve mAb stability and production titer.

SUMMARY

[0006] In a first aspect, provided herein are methods of increasing stability of a first antibody, comprising substituting glycine, alanine, or serine at heavy chain position 56 (AHo numbering) to create a second antibody, wherein the second antibody is more stable than the unsubstituted first antibody. For example, glycine or serine may be substituted at heavy chain position 56. For example, glycine or alanine may be substituted at heavy chain position 56. For example, glycine may be substituted at heavy chain position 56.

[0007] In a second aspect, provided herein are methods of increasing stability of a first antibody, comprising substituting a hydrophobic amino acid at heavy chain position 80 (AHo numbering) of the first antibody to create a second antibody, wherein the second antibody is more stable than the unsubstituted first antibody. Examples of hydrophobic amino acid residues include alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, tyrosine, and valine. By way of example, the hydrophobic amino acid residue can comprise or consist of alanine, isoleucine, phenylalanine, leucine, methionine, or valine. By way of example, the hydrophobic amino acid residue can comprise or consist of phenylalanine, leucine, or valine.

[0008] In a third aspect, provided herein are methods of increasing stability of a first antibody, comprising substituting alanine, phenylalanine, isoleucine, leucine, methionine, threonine, or valine at heavy chain position 80 (AHo numbering) of the first antibody to create a second antibody, wherein the second antibody is more stable than the unsubstituted first antibody. For example, phenylalanine, leucine, or valine may be substituted at heavy chain position 80. For example, isoleucine or methionine may be substituted at heavy chain position 80. For example, isoleucine may be substituted at heavy chain position 80. For example, methionine may be substituted at heavy chain position 80.

[0009] In sub-aspects of these first three aspects, the increased stability of the second antibody is demonstrated by at least one selected from the group consisting of an increase in titer during cell culture, increased yield from cell culture, increased purity after purification, a reduction in high molecular weight species, an increased melting point temperature, an increased temperature of aggregation, and an increased temperature of the onset of melting. In some sub-aspects, the increase in titer is measured by the rate of binding to a protein A coated probe tip using an Octet Forte Bio Instrument; and/or the increased yield is measured by protein A or protein G capture; and/or the increased purity is measured by SEC of purified protein; and/or the reduction in high molecular weight species is measured by size-exclusion chromatography (SEC) and the area under the curve of each peak for each molecular weight; and/or the increased melting point temperature is measured by differential scanning fluorimetry (DSF) or differential scanning calorimetry (DSC); and/or the increased temperature of aggregation is measured by DSF; and/or the increased temperature of the onset of melting is measured by DSF.

[0010] In some sub-aspects of the first aspect, the second antibody is further substituted with a hydrophobic amino acid residue at heavy chain position 80 (AHo numbering). For example, the hydrophobic amino acid residue can comprise or consist of: alanine, isoleucine, phenylalanine, leucine, methionine, or valine. For example, the hydrophobic amino acid residue can be selected from the group consisting of: phenylalanine, leucine, and valine. In some sub-aspects of the first aspect, the second antibody is further substituted with methionine at position 80 (AHo numbering), or, alternatively, the second antibody is further substituted with isoleucine at position 80 (AHo numbering). In some sub-aspects of the first aspect, the second antibody is further substituted with alanine, phenylalanine, isoleucine, leucine, methionine, threonine, or valine at position 80 (AHo numbering). In some sub-aspects of the first aspect, the second antibody is further substituted with phenylalanine, leucine, or valine at position 80 (AHo numbering).

[0011] In some sub-aspects of the second and third aspects, the second antibody is further substituted with glycine, alanine, or serine at position 56 (AHo numbering). In some sub-aspects of the second and third aspects, the second antibody is further substituted with glycine or alanine at position 56 (AHo numbering). In some sub-aspects of the second and third aspects, the second antibody is further substituted with glycine or serine at position 56 (AHo numbering). In some sub-aspects of the second and third aspects, the second antibody is further substituted with glycine at position 56 (AHo numbering).

[0012] In these first three aspects, the first antibody is a monoclonal antibody, such as, for example, a human, or humanized, antibody. Furthermore, the first antibody is an IgG antibody, such as an IgG antibody selected from the group consisting of an IgG1, IgG2, IgG3, and IgG4 antibody. That is, the IgG antibody can be an IgG1 antibody, the